

Influence of different nitrogen sources and levels on ion content of cabbage (*Brassica oleracea* var. *capitata*)

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Abstract The objective of this study was to determine the effects of different nitrogen (N) sources and levels on ion content in cabbage (*Brassica oleracea* var. *capitata* ‘Yalova-1’). In the process of ion uptake by plants, electro-neutrality is maintained both by the plant and the nutrient medium in which the plant is grown. N fertiliser not only affects yield but also the quality of the plant. Nitrates convert to nitrite in plant tissues which may cause health problems in infants and also form carcinogenic substances. Higher doses of nitrites change haemoglobin to methaemoglobin and this inhibits the transport of blood oxygen in the human body. Increased rates of N fertilisers also cause the accumulation of oxalic acid in vegetables. Oxalic acid causes acute toxicity if taken with calcium (Ca) and this forms stones in the kidney. Cabbage plants were grown in field conditions with five N fertiliser types, four doses and three replications. The treatments of N consisted of: no added fertiliser; 100, 200, and 400 kg N ha⁻¹ as potassium nitrate, ammonium nitrate, urea, ammonium sulphate, and farmyard manure. The results demonstrate that plant head weight was generally greater when mineral fertiliser rather than organic fertiliser was supplied to plants. In the

400 kg N ha⁻¹ treatment as potassium nitrate, ammonium nitrate, ammonium sulphate, urea, and farmyard manure application, plant yields were 66%, 61%, 40%, 49%, and 44% higher than with no added fertiliser, respectively. When the data were subjected to multiple regression analysis, effects of ammonium nitrate fertiliser application at 342 kg N ha⁻¹ on plant yields (3333 g plant⁻¹) were higher than that of other fertiliser applications. With increasing NO₃⁻ nutrition, the bulk of anion charge appeared as organic anion accumulation in the plants. The increase in organic anion accumulation was paralleled by an increase in cation concentration (K⁺, Ca⁺², Mg⁺², Na⁺). Total inorganic anion levels (NO₃⁻, SO₄²⁻, H₂PO₄⁻, Cl⁻) were relatively constant. The effects of increasing NO₃⁻ nutrition in stimulating organic anion accumulation such as glutamate, malate, and oxalate were far greater than ammonium and the other nutrient forms. Nitrate content of plants increased with the increasing N application, especially with nitrate fertiliser. The increases observed in the plant were highest in the plots with potassium nitrate applied and lowest in plots with no added fertiliser treatment. The results also indicated that farmyard manure was much more suitable than mineral fertilisers for plant quality according to lower total N, nitrate, and oxalic acid contents of cabbage plants. However, ammonium sulphate application at the rate of 250 kg N ha⁻¹ was the most suitable fertiliser application rate for plant quality according to lower risk yields of plants (2650 g plant⁻¹) for human nutrition.

Keywords nitrogen fertilisation; organic acid; nitrate content; cabbage; yield

INTRODUCTION

Cabbage (*Brassica oleracea* var. *capitata*) yield can be increased with proper fertilisation, together with other agricultural practices. Sufficient amounts of soil nitrogen (N) and fertiliser are very important for increasing yield. Increasing N applications usually

increases total fresh weight (FW) production, total N and nitrate concentrations in the plants, and decreases dry matter (DM) content (Kraxner et al. 1988; Guttormsen 1996). N fertiliser affects yield as well as the quality of the plant. Adding N fertiliser to soil affects the concentration in the soil solution of the added ions, and those of other ions already present in the soil. Large applications of easily available N at the time of planting are known to increase shoot:root ratios, and thus increase the susceptibility to physiological disorders later in the season (Marschner 1995). Brassicas, such as cabbages, are often considered as calscicoles, which accumulate free calcium ions (Ca^{+2}) (White & Broadley 2003), but restricted root growth is an important factor shown to reduce Ca uptake and induce tipburn (Aloni 1986; Magnusson 2004). This secondary effect is caused by ion exchange and electrochemical equilibrium processes (Sisson et al. 1991; Davies & Sylvester-Bradley 1995; Yanai et al. 1998).

Soil organic matter, and especially its humified fractions, the humic substances of organic fertiliser, are universally recognised to be among the most reactive soil components. They contribute substantially to maintaining global soil fertility status for agricultural production, and also to soil protection from degradation and contamination (Senesi & Loffredo 1999; Deng & Dixon 2002).

In the process of ion uptake by whole plants, electro-neutrality is maintained both in the plant and the nutrient medium in which the plant is grown. As plant roots absorb nutrient ions at different rates, electro-neutrality is achieved within the plant by the accumulation or degradation of non-volatile organic acids, which at the neutral to slightly acid pH of most cell vacuoles are dissociated and present mainly as anions (Imas et al. 1997a,b). The presence of respiratory CO_2 in the root medium implies that H^+ co-transport in the case of predominant anion content and OH^- excretion may appear in the nutrient medium as HCO_3^- . In plants supplied with non-N containing salts, short-term experiments indicated that when cations were taken up more rapidly than anions, stoichiometrical amounts of non-volatile organic anions were accumulated, corresponding to the excess cations absorbed (Van Beusichem et al. 1987; Marschner 1995; Aslam et al. 2001).

The forms of N content and N assimilation are of special importance in the formation of intracellular H^+ and OH^- . In the cells supplied with NO_3^- -N, the reduction of NO_3^- results in the production of one OH^- (Kirkby & Knith 1977; Kirkby & Armstrong 1980).

$\text{NO}_3^- + 8\text{H}^+ + 8\text{e}^- \rightarrow \text{NH}_3 + 2\text{H}_2\text{O} + \text{OH}^-$ as the result of NO_3^- assimilation, when NH_4^+ is the source, NH_4^+ content leads to H^+ production, $\text{NH}_4^+ + \text{NH}_3 + \text{H}^+$ which is almost quantitatively released into rhizosphere. As a consequence, acidification of the rhizosphere will reduce cation content, leading to decreased formation of organic acid NH_4^+ nutrition. NH_4^+ assimilation is thought to slightly decrease cellular pH.

The extreme difference in behaviour between NO_3^- and NH_4^+ assimilation in intracellular OH^- or H^+ production accounts for the well known observation that concentrations of organic acid anions are always higher in plants fed NO_3^- than NH_4^+ (Arnozis & Findenegg 1986; Allen & Raven 1987). In the removal of H^+ or OH^- from the cell to the external medium associated with these two forms of N content and assimilation, electro-neutrality is again maintained by a difference in the rates of cation and anion content. A greater content of cations, including NH_4^+ , is associated with the net H^+ efflux characteristic of NH_4^+ nutrition, whereas the greater content of anions that usually occurs in plants fed NO_3^- is counterbalanced by an equivalent net OH^- efflux (Van Beusichem 1981; Van Beusichem & Neeteson 1982).

The objectives of this study were to determine the effects of different N sources with NO_3^- and NH_4^+ , and N levels on plant yield, ion content, and organic anion accumulation in cabbage plants under field conditions.

MATERIALS AND METHODS

The experiment was carried out from 2000 to 2001 in the Agricultural Farmland of Ataturk University, Erzurum, Turkey ($39^\circ 55' \text{N}$, $41^\circ 61' \text{E}$). Soil properties of the experimental fields were: soil texture loamy, CEC $38.24 \text{ cmol}_c \text{ kg}^{-1}$, total N 0.11%, soil reaction neutral pH 7.05 in H_2O , organic matter content 2.20%, CaCO_3 0.80% and plant available phosphorus 3.80 mg kg^{-1} , exchangeable Ca $25.2 \text{ cmol}_c \text{ kg}^{-1}$, Mg $5.0 \text{ cmol}_c \text{ kg}^{-1}$, K $2.30 \text{ cmol}_c \text{ kg}^{-1}$, Na $0.20 \text{ cmol}_c \text{ kg}^{-1}$, available Fe 3.80 mg kg^{-1} , Mn 8 mg kg^{-1} , Zn 2.30 mg kg^{-1} , Cu 1.10 mg kg^{-1} , B 0.78 mg kg^{-1} , and Mo 0.13 mg kg^{-1} . Electrical conductivity was 1.05 dS m^{-1} (Page et al. 1982).

Plant cultivation

The experiments were laid out as randomised block designs with four rates of N application (without added fertiliser; 100, 200, and 400 kg N ha^{-1}), five

N sources (potassium nitrate, ammonium nitrate, urea, ammonium sulphate, and farmyard manure), three replicates (54 cabbage plants in each plot). Before planting, the experimental fields were sampled to determine the amount of mineral N in the soil layers 0–30 cm (21 kg N ha⁻¹) and 30–60 cm (0.2 kg N ha⁻¹). Triple superphosphate (120 kg P₂O₅ ha⁻¹) and potassium sulphate (150 kg K₂O ha⁻¹) were applied, broadcast as basal fertilisers. N was applied by band placement. Farmyard manure was analysed to determine mineral N content (0.476% N). Farmyard manure application was adjusted to provide similar levels of N as the chemical fertilisers. With band placement, the fertiliser was placed by hand in a hand drawn furrow, 5 cm on the side of the row and 5 cm deep, after which the furrow was closed again (Everaarts 1993). One week before planting, the soil in the plots was watered to volumetric moisture content of 0.28 cm³ cm⁻³. All plots were watered to maintain this soil moisture throughout the growing period.

Sampling and analytical methods

At heading time, the petiole and the midrib of the least mature full open foliage leaves of 54 (18 × 3 = 54 plant in each plot) plants were sampled (Berard et al. 1990). At harvest time, weight of heads and total yields were determined. The tissues sampled at heading were oven-dried at 68°C for 48 h and ground. Total H₂PO₄⁻, K⁺, Ca⁺², and Mg⁺² were determined after wet digestion of dried and ground subsamples in a H₂SO₄-Se-salicylic acid mixture with three additions of H₂O₂. In the diluted digests, P was measured spectrophotometrically by the indophenol-blue method after reaction with ascorbic acid. K and Ca⁺² were determined by flame photometry, and Mg⁺² by atomic absorption spectrometry (AOAC 1990).

The nitrate content in the leaves was estimated as described by Agbaria et al. (1996). One-hundred mg FW of leaf samples were extracted for 60 min in deionised water at 45°C. After centrifugation at 6000 rpm for 15 min, 200 µl of the supernatant was incubated at ambient temperature (24°C) with 0.8 ml 5% salicylic acid in concentrated sulphuric acid for 20 min. After 12 min, the samples were cooled to ambient temperature and the coloration was measured spectrophotometrically at 410 nm. The nitrate content was determined using a standard curve established with a solution of KNO₃.

For the determination of Cl⁻ and SO₄⁻², 1 g dried and ground subsamples were extracted with demineralised water. In the filtered extracts, Cl⁻ was

determined calorimetrically with an Ag anode at constant current, and SO₄⁻² turbidimetrically. Fresh leaf and midrib samples (0.5 g) were crushed with cold phosphate buffer (50 mM KH₂PO₄, pH 7.0) and centrifuged at 12 000 rpm for 15 min. Amino acids and ammonium were assayed on an amino acid analyser (ion exchange procedure with lithium citrate buffers) (Van Beusichem & Neeteson 1982).

Excess anion (A) content was calculated from A–C values related stoichiometrically to amounts of carboxylates and excess cation (C) content was calculated from C–A values (Dijkshoorn et al. 1968; Houba et al. 1971).

Statistical analysis

All data were subjected to two-way analysis of variance (ANOVA), separated by Duncan's multiple range test, and to multiple regression analysis to evaluate the effects of fertiliser application rate. Statistical analyses were performed using (SAS) statistical software (SAS 1982).

RESULTS AND DISCUSSION

Effects of different forms of N nutrition and fertiliser on plant dry matter (DM) yields, content of ions, and organic anion accumulation except for Cl⁻ were statistically significant (Tables 1 and 2). As expected, increasing N from deficiency levels to high levels exerted a very marked influence. Fertiliser type and doses significantly influenced head weight and ion contents of cabbage plants. The plant head weight was generally greater in the plants supplied with mineral fertiliser than organic fertiliser in the 400 N ha⁻¹ treatment as potassium nitrate, ammonium nitrate, ammonium sulphate, urea, and farmyard manure application. Plant yields were 66%, 61%, 40%, 49%, and 44% higher than without added fertiliser treatment, respectively. When the data were subjected to multiple regression analysis, the highest yield (3333 g plant⁻¹) was determined in the ammonium nitrate fertiliser supplied to plants in the 342 kg N ha⁻¹ ($Y = -0.0186X^2 + 12.73x + 1147.1, R^2 = 0.95$) treatment, compared with other treatments such as potassium nitrate (3288 g plant⁻¹ (389 kg N ha⁻¹, $Y = -0.0147x^2 + 11.463x + 1053.7, R^2 = 0.98$)); ammonium sulphate (2473 g plant⁻¹ (272 kg N ha⁻¹, $Y = -0.0156x^2 + 8.5153x + 1311.1, R^2 = 0.99$)); urea (2604 g plant⁻¹ (360 kg N ha⁻¹, $Y = -0.0102x^2 + 7.356x + 1278, R^2 = 0.98$)); and farmyard manure application (2216 g plant⁻¹ (3403 kg ha⁻¹, $Y = -0.002x^2 + 3.4023x + 1270.5, R^2 = 0.99$)), respectively.

Table 1 Results of ANOVA for head weight, cation, and anion uptake in cabbage (*Brassica oleracea*) plants.

Sources of variance	d.f.	Head weight	P level	Potassium	P level	Ammonium	P level	Calcium	P level	Magnesium	P level
Year (Y)	1	8.89	0.521	5.84	0.412	1.18	0.621	1.38	0.330	8.68	0.421
Fertiliser type (FT)	4	85.11	0.008	23.58	0.006	21.54	0.0011	126.43	0.0005	2.93	0.0005
Y × FT	4	99.55	0.009	2.19	0.047	9.47	0.0007	13.40	0.0009	3.41	0.0002
Fertiliser dose (FD)	3	1091.13	0.0007	194.65	0.0005	15.58	0.004	300.78	0.0001	2.69	0.0006
Y × FD	3	17.59	0.004	10.16	0.003	9.79	0.0003	35.89	0.003	0.41	0.624
FT × FD	12	23.91	0.006	4.9	0.001	7.18	0.0002	32.96	0.001	1.01	0.512
Y × FT × FD	12	8.26	0.002	1.12	0.123	1.18	0.206	2.84	0.002	1.29	0.086
Error	80										

Sources of variance	d.f.	Chlorine	P level	Nitrate	P level	Sulphate	P level	Phosphate	P level
Year (Y)	1	0.64	0.124	23.55	0.068	1.31	0.082	1.24	0.241
Fertiliser type (FT)	4	5.19	0.072	370.91	0.0001	20.84	0.0004	80.87	0.006
Y × FT	4	6.55	0.065	5.55	0.001	6.59	0.001	7.82	0.002
Fertiliser dose (FD)	3	5.49	0.071	4938.7	0.00004	23.71	0.008	172.81	0.0005
Y × FD	3	1.11	0.113	1.08	0.132	0.96	0.213	1.12	0.175
FT × FD	12	1.18	0.118	113.47	0.0002	2.22	0.003	17.78	0.002
Y × FT × FD	12	1.44	0.126	2.33	0.079	2.32	0.003	3.06	0.010
Error	80								

Excess anion content (EAC) calculated from total anions (A) and total cation (C) values related stoichiometrically to amounts of carboxylates and excess cation content (ECC) were calculated from C–A values (Dijkshoorn et al. 1968; Houba et al. 1971). Expressed on the basis of DM production, the plants fed NO₃⁻ caused substantially more cations and accumulated considerably more organic anions (C–A) than the plants fed NH₄⁺. For example, use of KNO₃ fertiliser produced greater head weight than (NH₄)₂SO₄ (13%), and it had more cation contents such as K⁺ (26% more), Ca⁺² (4% more), Mg⁺² (6% more), and accumulated more organic anions (84% more) than the (NH₄)₂SO₄ fertiliser. Content of inorganic anions such as PO₄⁻³ (6% more) and SO₄⁻² (23.21% more) was lower than that of the (NH₄)₂SO₄ fertiliser (Table 3). The increase in organic acid anion accumulation from the N deficiency threshold level of no added fertiliser to 400 kg ha⁻¹ treatment was paralleled by an increase in cation content (Table 4). This is reflected in the fairly constant level of inorganic anions making up the balance between cation and organic anions above the N deficiency threshold. At lower levels of nitrate nutrition, organic acid anion accumulation was low and inorganic anions were thus qualitatively more important in balancing inorganic cations. This might be because of the preserved electro-neutrality during nitrate assimilation (Kirkby & Armstrong 1980; Louahlina et al. 2000).

Increasing the level of nitrate nutrition stimulated DM production. The enhanced growth was accompanied by an increase in organic anion concentration in the plants and a parallel increase in cation content. The difference in ion content between treatments largely resulted from a disparity in N content. The content of K⁺, Ca⁺², and Mg⁺² were stimulated at the higher level of nitrate nutrition whereas Cl⁻ content was enhanced at the lower nitrate level. These synergistic and antagonistic effects in ion balance have often been observed in plants grown under different nitrate regimes (Kirkby & Knight 1977).

The content of sulphate inorganic anions in nitrate nutrition was lower than the ammonium nutrition. These results agreed with other findings comparing NO₃⁻ and NH₄⁺ nutrition (Van Beusichem et al. 1985; Allen & Raven 1987; Van Beusichem et al. 1987).

Table 2 Results of ANOVA for organic acid contents of cabbage (*Brassica oleracea*) plants.

Sources of variance	d.f.	Aspartate	P level	Glutamate	P level	Citrate	P level	Malate	P level	Succinate	P level
Year (Y)	1	4.68	0.241	2.37	0.128	2.15	0.162	0.21	0.284	18.86	0.072
Fertiliser type (FT)	4	0.73	0.326	5.01	0.014	11.76	0.024	9.51	0.032	1.82	0.124
Y × FT	4	1.38	0.124	1.20	0.269	7.2	0.241	3.37	0.084	2.01	0.103
Fertiliser dose (FD)	3	12.19	0.005	88.16	0.003	141.7	0.0007	6.69	0.041	4.04	0.096
Y × FD	3	1.39	0.265	1.29	0.143	6.64	0.189	1.14	0.110	1.27	0.128
FT × FD	12	1.36	0.250	1.33	0.127	2.07	0.127	1.08	0.102	2.60	0.110
Y × FT × FD	12	0.88	0.310	0.42	0.218	2.23	0.162	0.39	0.184	1.34	0.130
Error	80										
Sources of variance	d.f.	Oxalate	P level	Formate	P level	Ascorbate	P level	Fumarate	P level		
Year (Y)	1	31.61	0.052	26.14	0.124	1.31	0.108	1.19	0.212		
Fertiliser type (FT)	4	94.73	0.001	22.19	0.118	11.32	0.096	1.40	0.231		
Y × FT	4	10.51	0.165	1.07	0.236	0.07	0.217	1.80	0.197		
Fertiliser dose (FD)	3	30.02	0.050	1.50	0.245	6.94	0.083	0.31	0.224		
Y × FD	3	3.58	0.100	0.99	0.266	1.12	0.116	0.39	0.236		
FT × FD	12	14.91	0.128	8.14	0.154	5.91	0.096	0.06	0.256		
Y × FT × FD	12	2.61	0.231	0.63	0.238	0.27	0.188	0.28	0.218		
Error	80										

Nitrate contents of plants increased with the increasing N application, especially with nitrate fertiliser forms. Application of the nitrate fertilisers enhanced plant nitrate content, but ammonium fertilisers decreased nitrate content. Effects of organic fertiliser on plant nitrate contents were lower than effects of mineral fertiliser. The increases observed were highest in plants supplied with potassium nitrate and lowest in plants with no added fertiliser. Nitrate absorbed by plant roots is available for translocation to the shoot, storage in vacuoles, and assimilation into reduced N products. The processes of nitrate content, translocation, and assimilation are interdependent and closely regulated in higher plants (Huber et al. 1996; Sivasankar & Oaks 1996). High oxalic acid content in plants may reduce plant quality and this causes a reductions in its consumption both by humans and animals because of health risks. Oxalic acid content of plants increased with increasing content of plant nitrate by nitrate fertiliser application. High amounts of nitrate and oxalic acid may cause health problems. Oxalate in plant foods impacts human health in at least two significant ways (Libert & Franceschi 1987; Holmes & Assimos 1998). First, oxalate is an anti-nutrient in that it renders Ca unavailable for nutritional absorption by humans (Heaney et al. 1988; Weaver et al. 1997). This issue of Ca bioavailability is important, given the reliance of populations around the world on plant foods as their main sources of Ca and other minerals. Second, soluble oxalates have sometimes been shown to pose a hazard when ingested by grazing livestock (Jamas 1999) and humans (Holmes & Assimos 1998; Chen et al. 2001; Bhandari et al. 2002). Oxalic acid may also cause acute toxicity if taken with Ca, and precipitate to form kidneys stones (Vogtmann & Biedermann 1985; Margerath 1989; Walker 1990). So, ammonium sulphate is the most suitable chemical N fertiliser for plant quality according to nitrate and oxalic acid contents of cabbage plants. Farmyard manure is the most suitable organic fertiliser.

The influences of the level of N supply on organic acid accumulation are given in Table 4. ANOVA results indicated that fertiliser type and doses significantly influenced some organic acid content of plants (Table 2). The effects of increasing NO₃⁻ nutrition in stimulating organic anion accumulation such as glutamate, malate,

Table 3 Head weight, cation, and anion uptake by cabbage (*Brassica oleracea*) plants supplied with NO₃⁻ or NH₄⁺ as the nitrogen (N) source. Values are means of 54 plants in each plot. (WAF, without added fertiliser; OAA, organic anion accumulation; EAC, excess anion content; ECC, excess cation content.)

Form of N fertilisers: Doses (kg N ha ⁻¹): Head weight (g plant ⁻¹); 1103 d*	Potassium nitrate			Ammonium sulphate			Ammonium nitrate			Urea			Farmyard manure			
	WAF	100	200	WAF	100	200	WAF	100	200	WAF	100	200	WAF	100	200	400
Head weight (g plant ⁻¹); 1103 d*	1922 c	2858 b	3275 a	1328 c	1962 b	2425 a	2220 a	1250 c	1962 b	3160 a	3240 a	1313 c	1818 b	2410 a	2572 a	2301 a
Cations and anions (meq 100 g dry weight plants⁻¹)																
Potassium	52.4d	78.8c	91.8b	50.3b	60.5ab	69.7a	71.2a	56.4b	57.9b	64.1a	68.8a	55.6b	59.2b	63.3ab	75.9a	77.4a
Ammonium	0.9c	1.2c	2.3b	0.8d	3.68c	46.2b	53.8a	0.7d	5.3c	17.4b	22.6a	0.8c	3.4b	6.8a	8.3a	11.6a
Calcium	30.0b	37.0ab	40.5a	28.0b	33.5ab	40.5a	42.5a	30.0	33.5	32.0	30.0	28.0b	36.0ab	40.5a	45.0a	43.0a
Magnesium	25.8b	27.0ab	28.3a	24.1b	25.6ab	26.7a	28.7a	25.7b	27.0ab	28.6a	29.3a	24.2a	27.5ab	32.5a	34.2a	33.3a
Total cations (C)	105.3	146.3	164.4	103.2	156.4	183.1	196.2	112.8	123.7	142.1	150.7	108.6	126.1	143.1	163.4	165.3
Chloride	39.7	36.2	30.3	33.6	32.9	30.1	28.8	37.4	35.2	35.1	33.8a	37.5	39.2	38.3	33.8	30.2
Nitrate	1.8d	43.0c	64.9b	1.3d	4.8c	8.7b	16.1a	1.7d	12.2c	44.6b	59.8a	1.8c	6.9b	18.5a	20.1a	18.1a
Sulphate	41.9b	51.9b	61.2ab	48.6c	70.1b	82.5a	85.7a	48.1b	54.4ab	58.1a	63.1a	43.7b	50.7ab	55.6a	60.1a	81.8a
Phosphate	19.3b	21.3b	23.2b	17.3b	23.8ab	29.6a	27.1a	19.3c	30.2b	38.1a	39.9a	17.4b	19.9ab	20.2a	22.4a	23.8a
Total anions (A)	102.7	151.4	185.6	100.8	131.6	150.9	157.7	106.5	132.0	175.9	196.6	100.4	116.7	132.6	139.4	153.9
OAA	0.8	13.7	25.8	1.2	1.6	4.3	6.2	2.6	10.5	20.2	51.2	3.1	2.1	2.5	5.2	8.8
EAC (A-C)	-	5.1	11.2	23.6	-	-	-	-	8.3	33.8	45.9	-	-	-	-	11.4
ECC (C-A)	2.6	-	-	3.2	24.8	32.2	38.5	6.3	-	18.8	54	8.2	9.4	10.5	24.0	1.6
Anion assimilation	-	18.8	37.0	64.3	-	-	-	-	-	-	-	-	-	-	-	-
Net cation assimilation	1.8	-	-	2.0	23.2	27.9	22.3	8.9	-	5.1	7.3	13.0	19.8	5.0	-	-

*Values are the significances tested in rows.

Table 4 Organic acid composition of cabbage (*Brassica oleracea*) plants supplied with NO₃⁻ or NH₄⁺ as the nitrogen (N) source. Values are means of 54 plants in each plot. (WAF, without added fertiliser.)

Form of N fertilisers: Doses (kg N ha ⁻¹):	Potassium nitrate			Ammonium sulphate			Ammonium nitrate			Urea			Farmyard manure			
	WAF	100	200	WAF	100	200	WAF	100	200	WAF	100	200	WAF	100	200	400
Organic acids (meq 100 g dry weight plants⁻¹)*																
Aspartate	0.2b	0.4b	1.8a	2.0a	0.2b	0.4b	1.2a	0.5c	1.2b	1.6b	3.6a	0.3b	0.1c	0.4a	0.7b	1.3a
Glutamate	0.1d	6.8c	9.9b	12.3a	0.2	0.2	0.2	0.3c	3.2c	6.2b	20.2a	0.5a	0.3b	0.3b	0.6	0.8
Citrate	0.2b	0.3b	1.6a	1.8a	0.4b	0.9b	2.6a	0.4c	0.6c	1.1b	2.1a	0.3a	0.2a	0.2a	0.3	0.4
Malate	0.1d	4.3c	7.5b	15.5a	0.1	0.1	0.2	0.5d	2.9c	5.8b	14.2a	0.5a	0.2b	0.2b	0.3b	0.7ab
Succinate	0.0	0.2	0.4	1.1	0.0	0.0	0.1	0.2	0.2	0.3b	1.4	0.3	0.1	0.2	0.3	0.5
Oxalate	0.2d	1.2c	3.4b	5.9a	0.2	0.2	0.4	0.9d	1.7c	3.4	5.8a	1.2	1.3	1.4	3.4	4.4a
Formate	0.0	0.2	0.5	0.5	0.0	0.0	0.2	0.0	0.2	0.3	0.9	1.3	0.0	0.1	0.2	0.3
Ascorbate	0.0	0.1	0.4	0.6	0.0	0.0	0.1	0.2	0.0	0.2	1.2	0.0	0.0	0.0	0.1	0.3
Fumarate	0.0	0.2	0.3	0.8	0.0	0.0	0.1	0.2	0.2	0.3	1.4	0.0	0.0	0.0	0.3	0.4
Total organic anions	0.8	13.7	25.8	40.7	1.2	1.6	4.3	6.2	10.5	20.2	51.2	3.1	2.1	2.5	5.2	8.8

*Values are the significances tested in rows.

and oxalate were far greater than ammonium and the other nutrition forms, but as N supplied as ammonium promoted preferential syntheses of citrate. Increasing the level of N from the no added fertiliser to 400 kg N ha⁻¹ treatment was paralleled by increases in organic acid accumulation. Glutamate, malate, and oxalate organic acid accumulation of plants were higher in ammonium nitrate and potassium nitrate fertilisers than in the other fertilisers. Aspartate, succinate, formate, ascorbate, and fumarate contents of plants were similar in all fertiliser applications. Different reports on the effects of N availability on the biosynthesis of plant amino acids are found in the literature. Most data point to an increase in amino acids, as well as in the biological value of proteins, with N level in the soil increased up to the optimal value (Finc 1982). It is also known that agro-ecological conditions as well as plant genetic characteristics have a strong influence upon N content and chemical composition of plants. Most plants are not capable of reducing a high content of soil nitrates and incorporating them into amino compounds. So surplus nitrate often accumulates within the plants (Santamaria & Elia 1997; Elia et al. 1998), becoming a limiting factor for N metabolism, or it is stored in the form of amides (Whitehead 1995). Schuphan (1961) recorded a decrease in protein quality with intensified N application to leafy vegetables, accompanied by a simultaneous rise in nitrates.

Malate was the major organic acid anion accumulated in response to increasing levels of nitrate assimilation. Malic acid synthesis can result from the carboxylation of phosphoenol pyruvate (PEP) to oxalic acetic acid (OAA) which is then reduced to malate by the enzyme malic dehydrogenase. Jacoby & Laties (1971) have suggested that an increasing pH may raise the HCO₃⁻ level in the cytoplasm and thus induce carboxylation by PEP carboxylates. The basic feature of both these postulated mechanisms is that an increase in cellular pH induces malate accumulation. Nitrate reduction is also accompanied by an alkaline pH shift in plants (Caputo & Barneix 1997), which stimulates malate accumulation.

Close agreement obtained in cation-anion balances indicated that the major cations and anions should be considered. This confirms earlier work where the inorganic cations (K⁺, Ca⁺², Mg⁺², and Na⁺) were found to be largely balanced by organic acid anions together with NO₃⁻, PO₄⁻³, Cl⁻, and SO₄⁻² (Lorenz 1976). This balance infers that different cations compete for the bulk of anions charge in the plant and vice versa. This probably explains

the fact that when nitrate nutrition was inadequate, an increase in concentration of all the inorganic anions occurred to make up for the anion deficiency in plants. Similarly, at higher levels of nitrate nutrition the stimulation in cation content appeared to be largely non-specific. Similar results were reported by Ruiz & Romero (1999) and Majerowicz et al. (2000).

CONCLUSION

Sources of nitrogenous fertiliser types and N rates significantly affected yield, ion content, and distribution of cabbage. Application of the nitrate fertilisers enhanced plant nitrate content, but ammonium fertilisers decreased nitrate content. Effects of organic fertiliser on plant nitrate contents were lower with the mineral fertilisers. The sources of N should be taken into consideration in areas where intensive cropping is practiced and better quality products are sought. N fertiliser has indeed caused a significant increase in crop yield; it has also brought about some unfavourable results. We believe that differences in organic and inorganic ion content of plants receiving mineral and organic fertiliser cause variations in the composition of yield, quality, and ion balance in cabbage plants. Therefore, farmyard manure being organic and biodegradable is the most suitable fertiliser in sustainable agriculture and for plant quality according to nitrate and oxalic acid contents of plants. However, ammonium sulphate application at the rate of 250 kg N ha⁻¹ is the most suitable fertiliser application rate for plant quality according to yields of plants (2650 g plant⁻¹) for human nutrition. Further studies should be conducted into different organic and mineral fertilisers to evaluate their effects on edible vegetable crops for increasing plant quality and lowering the health risk for humans.

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